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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1936

By

FAITH STONE MILLER

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CHICAGO, ILLINOIS

Reprinted from
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SOME EFFECTS OF STRYCHNINE ON RECONSTITUTION IN EUPLANARIA DOROTOCEPHALA[1]

(Twenty-one figures)

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IN the reconstitution of pieces of *Euplanaria dorotocephala* head-forms ranging from normal through a graded series of differentially inhibited heads to complete acephaly develop. The frequency of the various head-forms in a given lot of pieces has been called "head frequency". It has been shown that head frequency varies in definite ways with length of piece, level of body from which it was taken, and physiological condition of the animal and that it can be modified by the use of various chemical and physical agents (Child, 1911*a*, 1911*b*, 1916; Behre, 1918; Buchanan, 1922; Hinrichs, 1924; Child and Watanabe, 1935; Rulon, 1936). Most agents used, however, have been depressants, and it was thought that additional evidence as to the factors involved in reconstitution might be obtained by the use of an agent which is known to have a very great stimulating effect on the nervous system of higher animals, namely, strychnine.

The effects of strychnine have been reported for species from the majority of the invertebrate phyla and for many vertebrates. Sherrington (1905), working on the frog, was the first to point out that in strychnine tetanus both muscles of an antagonistic pair contract simultaneously, and the inhibition which occurs in normal reflex movements is absent. The resulting position of the animal depends on the relative strength and leverage of the muscles. He showed that this change of inhibition into excitation is due to the action of the strychnine on the spinal cord. Cushney (1919) observed that strychnine lowers the threshold of response to stimuli and that the tetanus which is induced may be compared to the "start" reflex which normally occurs in response to unexpected sound or touch. Studies by the Lapiques (1913), Bremer and Rylant (1925), Cowan and Ing (1935), and Knoefel (1936) show that strychnine can decrease and completely block conduction in peripheral nerve fibers. The evidence indicates that this peripheral action is depression rather than stimulation.

Strychnine acts specifically to a high degree on the nervous system in the higher invertebrates and vertebrates and when applied centrally results in marked stimulation. However, with animals that have a primitive nervous system or no definite nervous organs, the effect is not distinguishable from that of an anesthetic (various Protozoa—Kritz, 1924; Swindle and Kritz, 1924; and certain coelenterates—Moore, 1917; Child, 1926; and unpublished data of my own on *Tubularia*). Kymograph records demonstrating stimulation after applying strychnine to the ganglia have been made on the octopus (Baglioni, 1905) and on *Limulus* heart (Carlson, 1906). Crozier and Pilz (1924) produced central nervous excitation in grasshoppers and caterpillars by injection and direct application to the thoracic ganglia. Observations of increased activity, loss of co-ordination, and simultaneous excitation of antagonistic muscles have been recorded for a cteno-

[1] The writer wishes to express her appreciation to Dr. C. M. Child, under whom this work was carried on, for his very helpful suggestions and criticisms.

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phore (Moore, 1936), a nemertean (Crozier, 1926), annelids (Knowlton and Moore, 1917; Crozier, 1922, 1926), molluscs (Moore, 1916, 1917; Crozier and Arey, 1919*a*, 1919*b*; Crozier, 1920), echinoderms (Pearse, 1909; Moore, 1916, 1918, 1920), and arthropods (Moore, 1913, 1916; Crozier, 1922; Blume, 1930; Clark and Wolf, 1932).

Very little is known of the nervous physiology of flatworms. Moore (1918) observed that in strychninized *Bdelloura* mechanical stimulation caused extension and activity instead of the normal reaction of contraction and adhesion to the substratum. Van Cleave (1929) was unable to alter head frequency in *Stenostomum leucops* with a number of chemical agents including strychnine sulphate although he did find higher head frequency in long pieces, freshly collected specimens, and at low temperatures.

Child (1914*a*, 1914*b*, 1916) and Child and Watanabe (1935) have shown that the degree of development of the head on a cut piece of *E. dorotocephala* depends on two factors: (1) the activity of the cells at the anterior cut surface and (2) an inhibiting factor arising from the posterior cut surface. In short pieces and at posterior levels of the anterior zooid the inhibiting factor is dominant, and consequently head frequency is low. In longer pieces and at anterior levels this factor is less effective. Observations of susceptibility (Child, 1914*a*), carbon dioxide production (Robbins and Child, 1920; Watanabe and Child, 1933), and oxygen consumption (Hyman, 1919*a*, 1919*b*, 1923) have shown that stimulation of the piece as a whole is greatly increased by cutting and decreases progressively with time after sectioning. However, head-frequency determinations on pieces in which the anterior cut was made at varying intervals after the posterior show that some inhibitory influence is present for as long as 3 or 4 days (Child and Watanabe, 1935). Since the stimulation caused by cutting as demonstrated by oxygen consumption and carbon dioxide production disappears in a few hours, it seems probable that the wound healing and early stages of tail regeneration are also concerned with the inhibition. Buchanan (1922, 1923) has suggested that this factor is mainly nervous in character and may be considered to arise from the severing of the nerve cords, and recent experiments by Child and Watanabe (1935) on *E. dorotocephala* and by Watanabe (1935*b*) on *E. tigrina* (=*E. maculata*)[2] indicate that this is probably the case.

With these facts in mind it was thought that investigation of the action of strychnine might contribute additional evidence upon this point. If the strychnine acts on *Euplanaria* as on higher animals, as a specific nervous stimulant, it might conceivably be expected to increase the effectiveness of the inhibiting factor and thus decrease the head frequency. Data from various lines of attack upon this problem will be presented, and their significance discussed.

MATERIAL AND METHODS

GENERAL PROCEDURE

Individuals of the species *E. dorotocephala* which were collected from spring-fed streams near Cary and Rockford, Illinois, and Valparaiso, Indiana, were used for these experiments. In order that they might be in as nearly as possible the same physiological condition they were kept in the laboratory for at least 2 weeks before use. Laboratory care consisted in feeding beef liver two or three times a week, changing the well water in which they were kept daily or on alternate days, and cleaning the pans of accumulated slime at frequent intervals. From a stock which had received at least 1 feeding in the

[2] Revision by Kenk (1935).

laboratory and then had been starved for 10 days to 2 weeks, worms of the same length and width which had not recently undergone fission were selected for use and were cut in pieces with a small knife. Cuts were made in water, and the pieces were transferred immediately into the solution. Cutting the worms in the solution was attempted, but it was found that they became so much more active that cutting pieces of uniform size was very difficult. Pieces from the same body-level were kept in lots of 20 or 25 in 200 cc. of fresh strychnine sulphate solution, made up in well water in finger bowls which were covered and kept in the dark. Concentrations used ranged from M/10,000 to M/500,000. Controls from the same lot of worms were cut and kept in well water under the same conditions as the treated series. Temperature was kept at about 18°–20° C. by thermostatic control of room temperature in winter and by keeping the finger bowls in covered pans surrounded by running water in summer. Exact temperatures were recorded daily in order to make sure that the fluctuations were not great. Observations were made after 10 days to 2 weeks and records made of head-forms; tailless, short, and normal tails; and types of bipolar forms. The head-forms which were described by Child (1911*b*, 1921, 1924) include: (1) normal (Fig. 1), (2) teratophthalmic, with the eyes connected or approximated and the head shape normal, (3) teratomorphic, with a single eye and the cephalic lobes pointing forward or fused in the mid-line, (4) anophthalmic, with an outgrowth of tissue but no eyes, and (5) acephalic, in which the anterior end healed but produced no outgrowth.

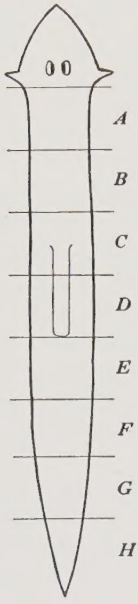


FIG. 1.—Body-levels of pieces used in head-frequency experiments. Each piece is 1/8 of post-cephalic length.

CALCULATION OF HEAD-FREQUENCY INDICES

In all cases only the living pieces were considered in making the graphs, and experiments containing a large number of deaths were discarded. The few experiments in which this was necessary were known to have been affected by rise in temperature, lethal concentration of the agent, or poor condition of the stock.

To obtain numerical values which could be expressed graphically as indices of head frequency the following formula was used (modified from Watanabe, 1935):

$$\frac{100n_5 + 80n_4 + 60n_3 + 40n_2 + 20n_1}{N}.$$

In this formula n_5–n_1 represent the numbers of pieces which develop normal, teratophthalmic, teratomorphic, anophthalmic, and acephalic forms, respectively, and N is the total number of pieces in the series. The coefficients 100, 80, 60, 40, and 20 are arbitrary values assigned to the various head-forms. This formula differs from that given by Watanabe only in that he includes a sixth term in the numerator, $0n_0$, to represent dead pieces. The evidence from the experiments here reported is that deaths often occurred after the heads were differentiated and that many, at least, of the pieces which died were not ace-

phalic but had normal heads. Consequently, dead pieces were omitted from the calculations of head frequency rather than being assigned a value (0) which implies that death was caused by a still greater degree of inhibition than that causing acephalics (value 20). Watanabe's formula, with this slight modification, was used in order that the indices should be comparable to those obtained by other workers in this laboratory. The omission of dead pieces does not greatly alter the values obtained since the frequencies of deaths were low in most cases. The distribution of deaths is discussed later. The scale used, then, ranges from 20 as a base line to represent acephalic forms to 100 for normal heads. In any case the values are purely arbitrary since the frequencies of the various head-forms in control lots show that the groups are not of equal weight. However, this type of graphing is believed to be adequate for comparative purposes.

In the majority of the experimental series bipolar forms appeared. For the purposes of graphing, all the bipolars at each body-level of a given series were combined, irrespective of the degree of development of the heads, and were expressed as percentages of the total number of living pieces at that level. In these groups pieces were also found which behaved like bipolars but had no posterior outgrowth or a short outgrowth which was classed as a short tail. Some of these were probably actually bipolar forms with acephalic or anophthalmic posterior ends. In the later experiments the direction of beat of the cilia on the ventral surface was observed by means of a carbon suspension as a test of the polarity of these forms. However, since this was not done in the early series, and in order to avoid any possibility of over-weighting the bipolar groups, these pieces were recorded as questionable, and only those which were definitely identified as bipolar bythe presence of two heads were included in making the graphs.

RATE OF EYE FORMATION

In order to determine whether or not the solutions were acting on the cells at the anterior cut surface, the time of appearance of eyespots was observed as an index of the rate of differentiation. Pieces were cut three-eighths of the postcephalic length with anterior cut ends at four levels of the anterior zooid. Thus, pieces used included *ABC*, *BCD*, *CDE*, and *DEF* (Fig. 1). Long pieces were used in order that all should develop normal heads. Watanabe (1935*a*) has shown that inhibited heads develop more slowly than normal and that pieces of this size are sufficiently long to produce 100 per cent normals. In five series three lots of 20 pieces each at each level were treated as follows: one lot in M/100,000 strychnine sulphate for 16 hours, another in M/450,000 throughout the experiment, and the third as control in well water, making a total of 100 pieces in each solution at each level. *ABC* and *DEF* pieces in the same solution were cut from the same animals, whereas *BCD* and *CDE* pieces were of necessity from different animals but from the same stock. Only four levels were used because of the fact that in more posterior pieces the eyes develop in the old tissue and cannot be distinguished accurately from body pigment in the early stages. Also, with only four levels, each series contained 240 pieces which was the maximum number that could be observed carefully. Temperatures were controlled as carefully as possible so that the five series would be comparable. Frequent readings showed that the fluctuations were not great and that, except for Series 3, the ranges were almost identical. The extremes of each group were as follows: Series 1, 18.5°–20°; Series 2, 18°–21°; Series 3, 20.5°–22.5°; Series 4, 18°–20.5°; and Series 5, 18.5°–21.5°. C.

Observations were made at 8-hour intervals, and numbers of pieces possessing eyes

were recorded. With the aid of a compound microscope with 16-mm. objective and 10× ocular, the eyespots were discernible as small pigment spots near the boundary of old and new tissue just beneath the ectoderm. By careful focusing and with the aid of strong transmitted light they could be distinguished from the body pigment by their deeper location.

From these data calculations were made of arithmetical means and standard deviations of each lot and of average standard deviations within lots. The statistical probability (P) that as great a difference might be produced by random sampling was calculated with the use of "Student's" formula ("Student," 1925). P was calculated for differences between strychnine-treated and control lots at each level and between the *ABC* and *DEF* pieces in each solution. Percentage values for developmental rates were obtained by taking the mean time of the *ABC* pieces in water as a standard and expressing all the other means relative to it.

EXPERIMENTAL DATA

GENERAL OBSERVATIONS

Intact worms put into strychnine solution (M/1,000–M/500,000) show greatly increased activity. This is at first manifested in both low and high concentrations by elongation of the head and of the body, lifting of the head from the substratum and waving it back and forth, rapid movement, and muscular tremors. Such worms will not feed, rheotaxis is not exhibited, and when turned over, their righting reaction is either very slow or absent. They are very easily stimulated to move by sudden increase in illumination or by slight agitation of the solution, but their locomotion seems poorly co-ordinated and they are easily dislodged from the substratum. Although they are highly motile, they do not show the tetanus, caused by touch or jarring, characteristic of strychnine action on higher forms. The typical reaction of untreated *Euplanaria* to jarring of the dish is a shortening of the body and firmer adhesion to the bottom. However, strychninized specimens react instead by elongation and increased motor activity. This is a case of "reversal of inhibition" similar to that described by Moore (1918) for the marine triclad *Bdelloura*. In lethal concentrations (M/1,000–M/200,000) the animals soon exhibit spasmodic contractions, violent coiling and twisting of the body, and inability to remain attached. Small splits appear in the body wall, and disintegration of the anterior zooid proceeds in general in an antero-posterior direction. Disintegration of the posterior zooid usually begins before that of the anterior zooid is complete, but the coiling of the worm makes the course of the disintegration difficult to follow. Muscular tremors are present until the cytolysis is complete.

Cut pieces show the same general effects with regard to elongation, increased activity, muscular contractions, and inability to remain attached. They disintegrate in lower concentrations than whole worms and also at lower temperatures than do the controls. The pieces are often found ventral side up with both ends raised—a position which seems to be a state of tonic contraction with the body bent by the strongest muscles. Split places, in pieces as well as in whole worms, often appear in high concentrations, but if not too extensive, will heal if the pieces are returned to well water. Pieces reconstituting in strychnine appear very elongate with long, narrow, and pointed heads. However, if these are placed into well water for a few hours, they return to normal shape, and the elongation is seen to be functional rather than morphological.

HEAD FREQUENCY

One-eighth pieces of 16-*mm. worms.*—To determine the effect on head frequency, experimental lots were exposed for varying lengths of time to various molecular concentrations of strychnine sulphate made up in well water. Preliminary experiments to determine barely sublethal concentrations showed that for 16-mm. worms M/450,000 for continuous exposure, M/100,000 for 16 hours, and M/300,000 for 3 days were the most effective. Worms were cut into eighths of the postcephalic length as shown in Figure 1.

The most noticeable effect in all the solutions is a very marked increase in head frequency (Table 1 and Figs. 2, 3, and 4). This increase occurs not only in pieces from the pharyngeal region (*C* and *D*), which also show higher head frequencies in anesthetics, but also in any lots of *A* and *B* pieces which show inhibited heads in the controls. In

TABLE 1

HEAD-FREQUENCY COUNTS IN FOUR LOTS, EACH CONTAINING 25 16-MM. WORMS

Series	Type of Head	A	B	C	D	E	F	G	H
Strychnine, M/450,000	Normal	80	63	38	39	49	48	73	99
	Teratophthalmic	1	21	22	26	23	30	16	.....
	Teratomorphic	.....	1	14	6	2	2	1	.....
	Anophthalmic	.....	.....	13	9	3	6	6	.....
	Acephalic	.....	.....	3	15	14	11	2	.....
	Bipolar	.....	.....	2	2	4	.....	.....	.....
	Dead	19	15	8	3	5	3	2	1
Index		99.8	94.6	78.0	74.2	80.6	80.2	91.0	100
Control	Normal	72	8	.....	.....	4	13	35	99
	Teratophthalmic	23	18	.....	.....	9	20	40	.....
	Teratomorphic	2	9	.....	.....	6	7	9	.....
	Anophthalmic	1	10	2	2	14	17	3	.....
	Acephalic	2	53	98	97	67	40	13	.....
	Bipolar	.....	.....	.....	.....	.....	.....	.....	.....
	Dead	.....	2	.....	1	.....	3	.....	1
Index		92.4	43.3	20.4	20.4	33.8	49.5	76.2	100

such lots head frequency is usually decreased by narcotics. The exact amount of increase in strychnine varies slightly with different lots of worms, but in practically every case the curve for the experimental group is above that for the control. However, in one series the *H* pieces (Fig. 1), which usually produce 100 per cent normals, developed a few teratophthalmics in water and still more in strychnine (Figs. 3 and 4). The curves for the controls vary slightly also in different lots of worms. In experiments in which the controls give rather high head-frequency indices the strychnine-treated series is very much higher.

Since strychnine-treated animals are easily activated by jarring and by sudden exposure to light, and it is known that mechanical stimulation will increase head frequency (Child, 1911*a*, 1920), it was thought that possibly some of the great increase noted in experimental lots might have been due, in part at least, to the agitation involved in changing the solutions and moving the dishes to make observations. Therefore, simultaneously with other series of continuous exposure to M/450,000 strychnine sulphate, which were

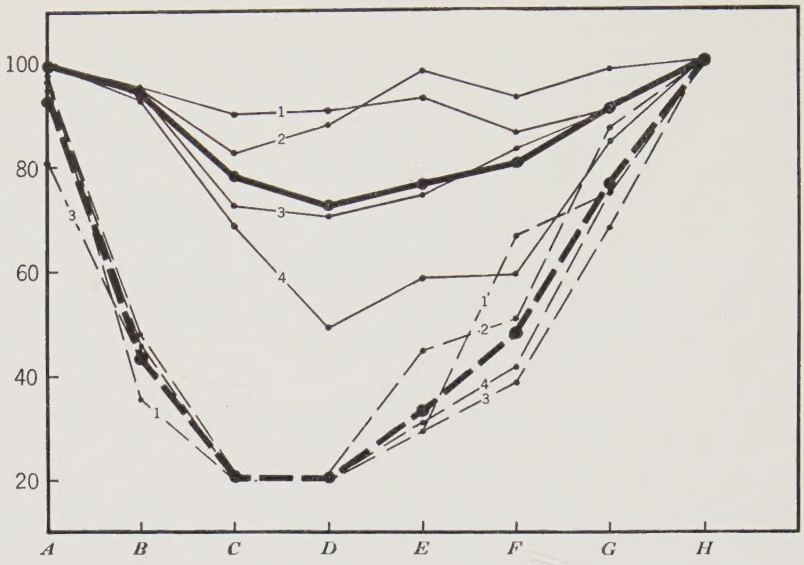


FIG. 2.—Head frequencies of 1/8 pieces of 16-mm. worms reconstituted in M/450,000 strychnine sulphate (continuous lines) and controls (broken lines). Corresponding experimental and control lots are indicated by numbers 1–4. Values obtained from combined counts (from Table 1) are shown by heavy lines. Ordinates are head-frequency indices and abscissae body-levels.

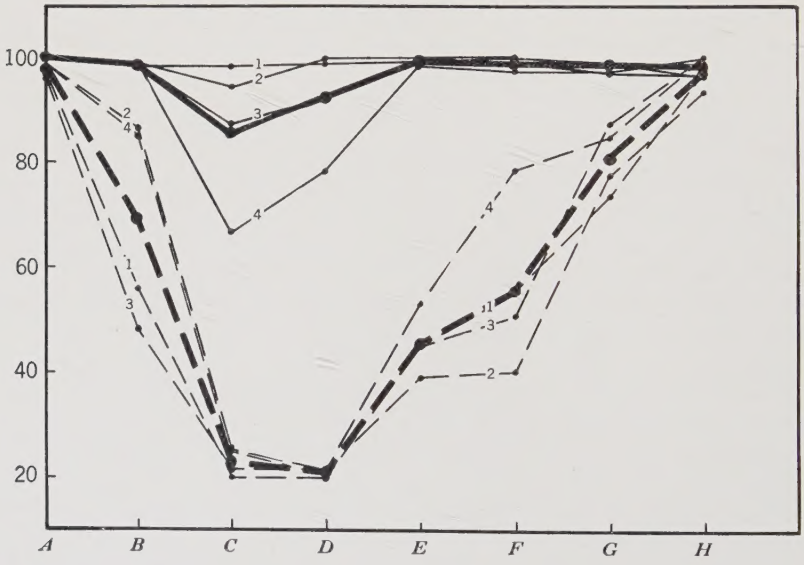


FIG. 3.—Head frequencies of 1/8 pieces of 16-mm. worms reconstituting in water after 3 days' exposure to M/300,000 strychnine sulphate (continuous lines) and controls (broken lines). Corresponding experimental and control lots are indicated by numers 1–4 and combined data by heavy lines. Ordinates are head-frequency indices and abscissae body-levels.

kept in finger bowls and treated in the usual manner, 50 experimental and 50 control worms were cut and placed in 1-liter Erlenmeyer flasks which were stoppered and placed in the dark. After 2 weeks, during which they were neither moved nor exposed to light, the types of heads were counted and head-frequency indices calculated. It was found that the values for both experimental and control lots which were not moved were slightly lower than those which were handled. However, those in strychnine were considerably higher than their corresponding controls, showing that mechanical stimulation played only a small part in the increase.

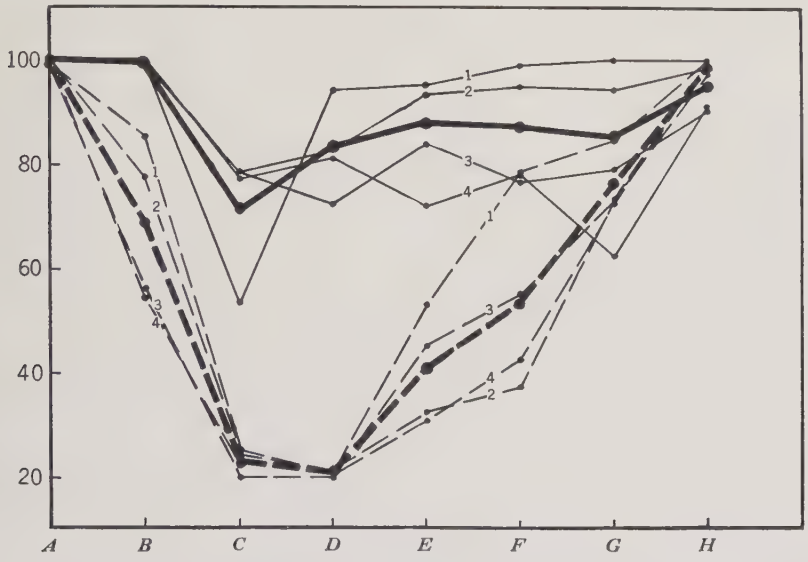


FIG. 4.—Head frequencies of 1/8 pieces of 16-mm. worms reconstituted after 16-hour exposure to M/100,000 strychnine sulphate (continuous lines) and controls (broken lines). Corresponding experimental and control lots are indicated by numbers 1–4 and combined data by heavy lines. Ordinates are head-frequency indices and abscissae body-levels.

In Table 1 are given the numbers of the various head-forms produced by continuous exposure to M/450,000 strychnine sulphate and also the head-frequency indices for each body-level. The latter are graphed in Figure 2. The curves shown in Figures 3 and 4 were obtained from similar lots of data. All three graphs (Figs. 2, 3, and 4) show the curves for each of the four pairs of experimental and control lots in light lines and the curves obtained from the combined data in heavy lines. The curves for corresponding experimental and control lots, which were cut at the same time from the same collection of worms, are designated by the same number.

The pieces exposed to M/300,000 for 3 days show the greatest increase (Fig. 3), although the curves for the treated lots in the other experiments are also conspicuously higher than those of the controls. Pieces exposed to M/100,000 give the most variable results (Fig. 4), although similar in general to those obtained with lower concentrations. Preliminary experiments, using only the anterior zooid of worms from the same collection and exposing the pieces for 8, 12, 18, and 20 hours, gave more uniform results than

repetitions of 16-hour exposures on pieces from different lots of worms even though the latter were kept under laboratory conditions and starved for the same length of time. This indicates that different lots of worms exhibit varying degrees of susceptibility to treatment. The percentage of bipolar forms produced is greatest in high concentrations. This point will be considered in the section on "Regeneration at Posterior Cut Surface."

Delayed posterior section.—It is known that the head frequency of very short pieces increases when the posterior cut is made at increasing intervals after the anterior cut (Child and Watanabe, 1935). A series of experiments to test the effect of such delay in strychnine as compared with the condition in water was made on pieces from the *E* level (Fig. 1). Four lots were run, each consisting of 25 pieces in strychnine and 25 in water

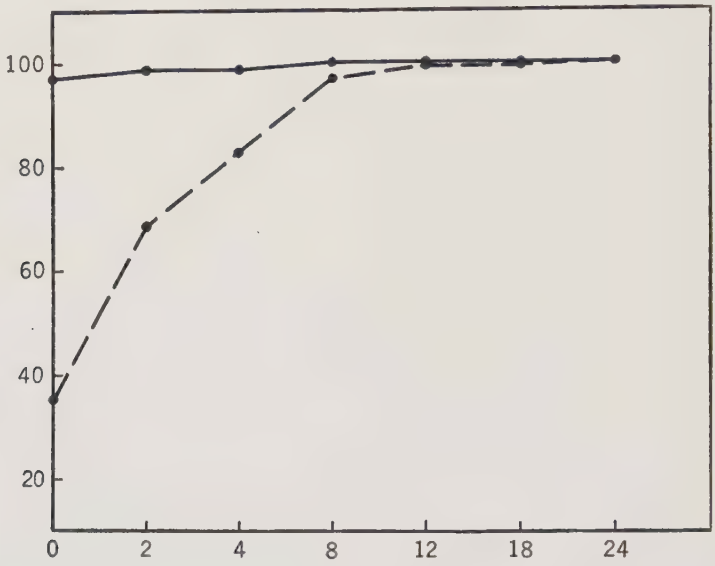


FIG. 5.—Head frequencies in M/500,000 strychnine sulphate (continuous line) and control (broken line) with delay of posterior section. Ordinates are head-frequency indices and abscissae intervals of delay in hours.

for each interval of delay. The first cut was made directly behind the mouth, where head frequency is lowest in controls, and the second cut was made 1 mm. behind the first at intervals of 0, 2, 4, 8, 12, 18, and 24 hours. Worms of 12–14 mm. were used, and the experimental series was exposed to M/500,000 strychnine sulphate. A slightly lower concentration than in the preceding experiments was used because of the fact that the pieces were smaller and from smaller worms and showed a higher susceptibility to the treatment.

Of the treated animals nearly all were normal after 0, 2, and 4 hours' delay and all were normal from 8 hours on; whereas the controls gave very low values at 0 hours, increasing to nearly all normal at 12 and 18 and all normal at 24 hours (Fig. 5). The large numbers of bipolar forms which occurred in these experiments will be described in the section on "Regeneration at Posterior Cut Surface."

Delayed anterior section.—A similar series was run with the anterior cut delayed. Child and Watanabe (1935) have shown that, for attainment of completely normal head fre-

quency, the delay must be very much longer than in the preceding series. The first cut was made a short distance behind the mouth, and the second made 1 mm. anterior to it at intervals of 0, 24, 48, 72, and 96 hours. Experimental lots were placed in M/500,000 strychnine sulphate and controls in well water.

In controls head frequency decreased from 0 to 24 hours and then increased with longer intervals of delay. This corresponds to the results obtained by Child and Watanabe (1935) on *E. dorotocephala* and Watanabe (1935*b*) on *E. tigrina*. In strychnine the results were similar to those for the delayed posterior-section experiment, i.e., high head frequency at all periods (Fig. 6).

REGENERATION AT POSTERIOR CUT SURFACE

In certain experiments large numbers of bipolar and tailless pieces were observed. Consequently, it was thought wise to consider the frequency of such developments at the posterior cut surface under a separate heading.

The numbers of bipolars appearing in 1/8 pieces are expressed as percentages of living pieces at each level in Figure 7. In all the solutions the *E* level showed the highest number, and the percentages corresponded to the concentration of the agent. The largest number (26.4 per cent at the *E* level) appeared in M/100,000, fewer (5.2 per cent) in M/300,000, still fewer (4.2 per cent) in M/450,000, and none in water at any level. Thus the high concentration is very much the most effective in producing bipolars. In Table 2 are recorded the types of outgrowth at the posterior cut surface in lots treated with M/100,000 and in their controls. This shows the distribution of various types of bipolar forms and also of tailless pieces and deaths. The form of the curve for bipolars (Fig. 7) is very regular, with a low peak at the *B* level and a high flat peak at the *E* level. This peak in *B* pieces also showed very distinctly in a few experiments with 1/16 pieces. Factors involved in this distribution will be considered in the discussion.

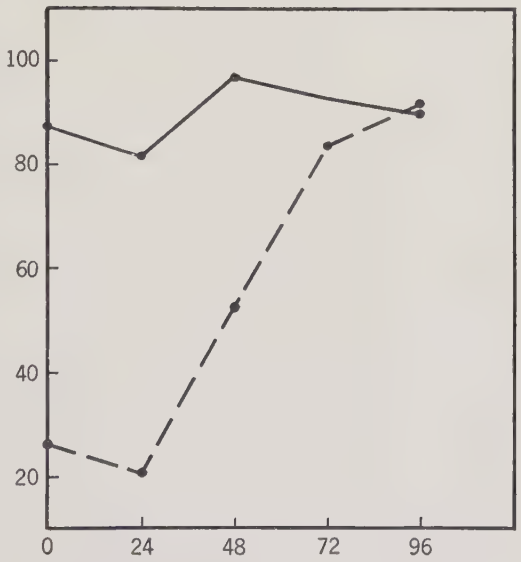


FIG. 6.—Head frequencies in M/500,000 strychnine sulphate (continuous line) and control (broken line) with delay of anterior section. Ordinates are head-frequency indices and abscissae intervals of delay in hours.

When the anterior cut was delayed, very few bipolars appeared. In strychnine several bipolars (18.2 per cent) and tailless (18.2 per cent) appeared after simultaneous sectioning whereas only one taillesss and no bipolars developed in water. After 24 hours' delay a few bipolars were produced in strychnine (8.1 per cent), but with subsequent delay all formed normal tails (Fig. 8).

The largest numbers of bipolar pieces, however, occurred in the experiments on delayed posterior section. With increasing intervals of delay the numbers of bipolars in

water increased from 1.1 per cent of living pieces after simultaneous anterior and posterior section to a maximum of 25.3 per cent after 12 hours' delay and 26.1 per cent after

TABLE 2

REGENERATION AT POSTERIOR CUT IN 1/8 PIECES OF 16-MM. WORMS
COMBINED DATA FROM FOUR LOTS EACH OF 25 WORMS

Series	Type of Outgrowth	A	B	C	D	E	F	G	H
Strychnine, M/100,000	Normal head	.....	5	2	7	6	9	5	.....
	Teratophthalmic	.....	.....	1	3	10	4	2	.....
	Teratomorphic	.....	2	1	5	5	3	4	.....
	Anophthalmic	.....	1	.....	2	2	5	1	.....
	No outgrowth	39	39	25	16	23	25	24	.....
	Short tail	6	15	12	17	12	17	22	.....
	Normal tail	47	28	45	23	29	30	37	94
	Dead	8	10	14	27	13	7	5	6
Control	Normal head	.....	.....	.....	.....	.....	.....	.....	.....
	Teratophthalmic	.....	.....	.....	.....	.....	.....	.....	.....
	Teratomorphic	.....	.....	.....	.....	.....	.....	.....	.....
	Anophthalmic	.....	.....	.....	.....	.....	.....	.....	.....
	No outgrowth	1	.....	.....	.....	4	3	.....	.....
	Short tail	.....	.....	.....	.....	7	.....	5	.....
	Normal tail	92	95	97	96	89	97	94	99
	Dead	7	5	3	4	.....	.....	1	1

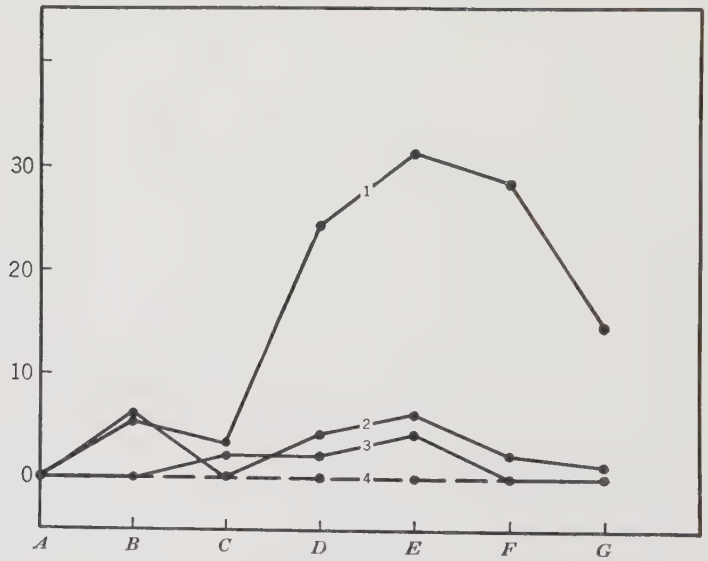


FIG. 7.—Percentages of bipolar forms produced in 1/8 pieces of 16-mm. worms in (1) M/100,000 strychnine sulphate, (2) M/300,000, (3) M/450,000, and (4) well-water control. Ordinates are bipolars in percentage of living pieces and abscissae body-levels.

18 hours (Table 3; Fig. 9). In strychnine large numbers appeared in all groups: 44.2 per cent after simultaneous section, 73.8 per cent after 12 hours' delay, and 38.8 per cent after 24 hours' delay.

All combinations of head-forms were produced (Figs. 10–19), but in most cases at least one head was normal, and the other cut surface produced a normal head more often than any other type. The more nearly normal head was taken as the originally anterior end.

Tailless pieces also occurred in large numbers in the 1/8 pieces exposed to M/100,000 (Table 2) and the delayed posterior-section experiments (Table 3). In the 1/8 pieces from different levels of the body these showed a differential distribution which was in general the reverse of that of the bipolars in the anterior zooid. In *A* pieces, where no bipolars appeared, the tailless were very numerous (42.4 per cent), but in the *D* and *E* pieces, where the bipolars were more numerous (23.3 per cent and 26.4 per cent), fewer tailless were found (21.9 per cent and 26.7 per cent).

Possible explanations for the occurrence of these forms are (1) direct inhibition of the cells at the posterior cut, (2) increased "scale of organization," or (3) incomplete reversal of polarity. In order to test the first hypothesis long pieces consisting of the head, *A*, and *B* were compared with *B* pieces alone in water and in strychnine. This level was used because many tailless pieces occurred here in strychnine experiments. No tailless pieces were formed in water in either long or short pieces, and none in strychnine in the long pieces, but several occurred in strychnine in the short pieces. This indicates that the action was not one of direct inhibition of the cells at the posterior cut surface but was related to the size of the piece and possibly also the presence of the head.

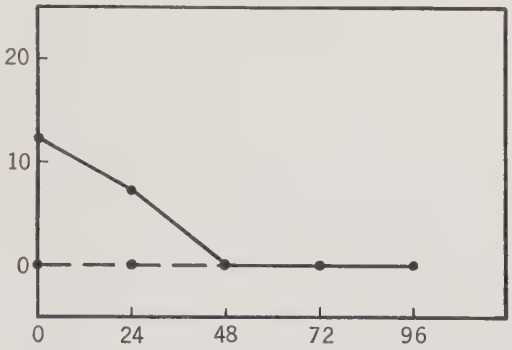


FIG. 8.—Percentages of bipolar forms with delay of anterior section in M/500,000 strychnine sulphate (continuous line) and control (broken line). Ordinates are bipolars in percentage of living pieces and abscissae intervals of delay in hours.

The position of the pharynx has been used as an index of what Child has called "scale of organization." He found (1911*a*, 1912, 1929) that in pieces regenerating in anesthetics the pharynx is smaller and closer to the head than in controls, and that with high temperature the reverse occurs. Observations of the position of the pharynx reconstituting in strychnine showed no significant alteration.

Reversal of polarity in questionable cases was determined by observing the behavior of the regenerated worms and the direction of beat of the cilia on their ventral surfaces. With the aid of a fine carbon suspension under a binocular microscope the direction of the current produced by ciliary action can be seen very easily (Rustia, 1925). In normal worms the cilia beat in an antero-posterior direction down the entire length of the worm, but in bipolar forms they beat from both ends toward the middle (Figs. 16–20).

In many of the pieces with no posterior new tissue the use of a carbon suspension demonstrated reversal of the ciliary beat at the healed surface. Such pieces often developed secondary tail-like lateral outgrowths of old tissue (Fig. 19). From their behavior, appearance, and ciliary reversal these were believed to have been actually bipolars with one completely inhibited head. Other pieces which produced no posterior new tissue, but which also had no ciliary reversal and no lateral outgrowths, were probably cases of inhibited tails (Fig. 20). Because of the difficulty of distinguishing accurately between these two types, and also because the ciliary beat was not observed in the earlier experi-

ments, none of these was included as bipolars in calculating the percentages of bipolar forms produced.

TABLE 3

REGENERATION AT POSTERIOR CUT AFTER VARYING INTERVALS OF DELAY OF POSTERIOR SECTION

Series	Type of Outgrowth	Number of Hours						
		0	2	4	8	12	18	24
Strychnine, M/500,000	Normal head	16	38	43	34	40	29	10
	Teratophthalmic	4	6	.....	1	.....	.....	.....
	Teratomorphic	11	6	3	5	7	7	11
	Anophthalmic	3	2	2	2	1	2	5
	No outgrowth	21	11	14	14	12	14	29
	Short tail	4	3	.....	1	1	2	3
	Normal tail	18	13	7	6	4	9	9
	Dead	23	21	31	37	35	37	33
Control	Normal head	.....	5	8	9	14	11	14
	Teratophthalmic	.....	.....	.....	.....	.....	.....	.....
	Teratomorphic	1	1	.....	1	5	4	3
	Anophthalmic	.....	.....	2	3	1	2	1
	No outgrowth	28	24	17	24	24	15	33
	Short tail	.....	4	2	3	8	8	3
	Normal tail	63	56	52	39	27	25	29
	Dead	8	10	19	21	21	35	17

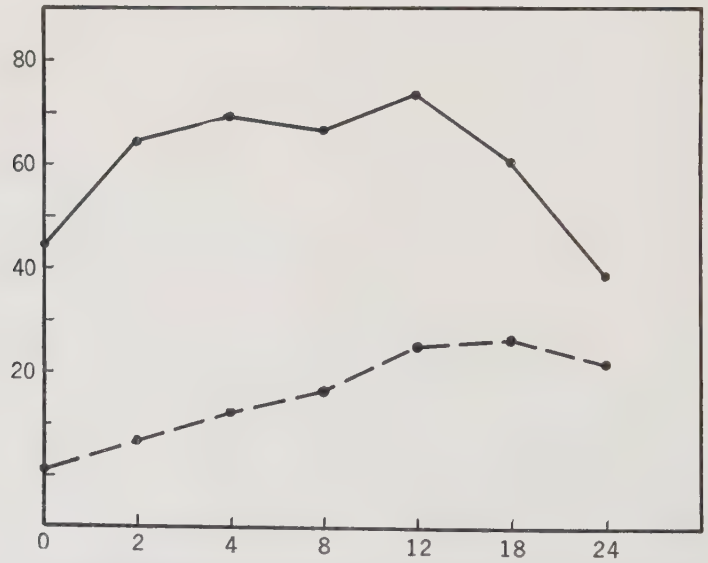
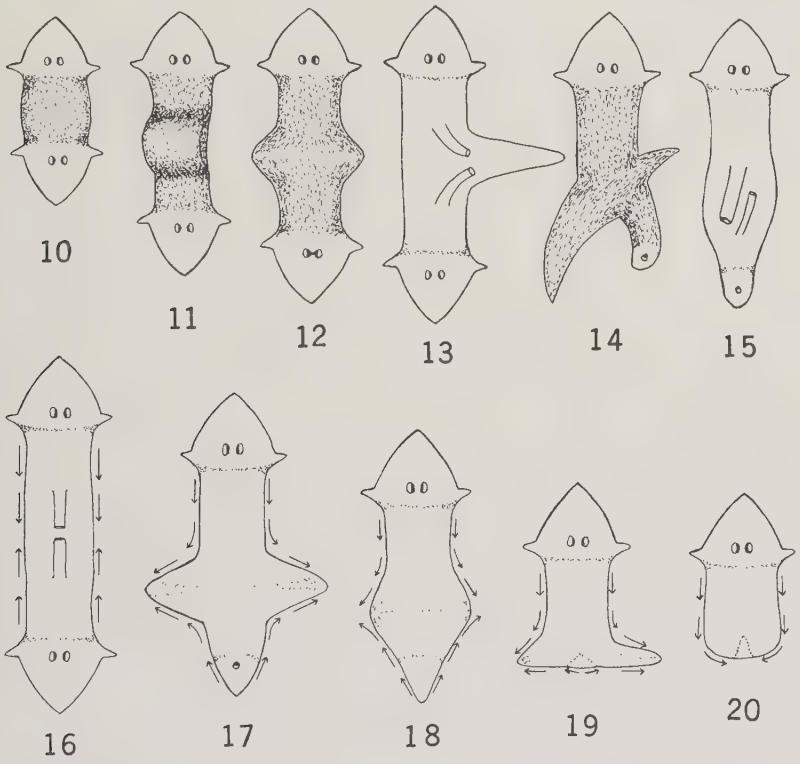


FIG. 9. –Percentages of bipolar forms with delay of posterior section in M/500,000 strychnine sulphate (continuous line) and control (broken line). Ordinates are bipolars in percentage of living pieces and abscissae intervals of delay in hours.

In the bipolar pieces, also, secondary lateral tails were often formed, sometimes on one side and sometimes on both and of varying lengths (Figs. 12–14, 17, 18). In cases in

which one head was normal and one inhibited, these outgrowths were at a greater distance from the normal than from the inhibited head, and one tail was often longer than the other (Fig. 14). A similar situation was observed with respect to the pharynges. In bipolar pieces with unequal heads the pharynges were located at a greater distance from the normal head, and the one determined in relation to the normal head was larger than that of the inhibited head (Fig. 15). Such cases demonstrate very clearly the difference



FIGS. 10–20.—Types of bipolar and tailless forms observed. Figures 12–14, 17–19, lateral tail-like outgrowths of old tissue. Figure 11, dorsal outgrowth of old tissue. Figures 12, 14, 15, 17, and 18, one normal and one inhibited head. Figures 13, 15, and 16, showing pharynges. Figures 16–19, reversal of direction of ciliary beat. Figures 19 and 20, no outgrowth at posterior cut surface.

in scale of organization determined by normal and by inhibited heads. Pharynx development was rather variable; none, one, two, or one with single attached and forked free ends were observed. The majority of the bipolar pieces possessed a dorsal outgrowth of old tissue (Fig. 11). In some this was so large in comparison to the ventral surface that the worms were unable to crawl normally but moved in circles, lying on one side with the two heads almost parallel.

DEATH FREQUENCY

Although the experiments were not planned to show the lethal effects of the agent, small numbers of dead pieces were observed in some solutions because of the fact that the concentrations dealt with were planned to be rarely sublethal, and different lots of

worms varied slightly in their degree of susceptibility. Deaths were highest in the delayed section experiments in which the size of the pieces was small. In the 1/8 pieces the distribution of deaths was seen to be opposite in the two extremes of concentration and exposure periods used (Fig. 21). In continuous exposure to M/450,000, deaths were most numerous in pieces from the anterior end and decreased posteriorly, whereas in 16-hour exposure to M/100,000, deaths increased to a high peak at the *D* level and then decreased. By the end of the 16 hours, when the pieces were returned to water, small split and disintegrating spots could be seen in many *D* and some *C* and *E* pieces. Some of these pieces died later, but the majority recovered.

After 10 days to 2 weeks, when the types of anterior and posterior regeneration were being recorded, pieces were frequently found with disintegrating areas in the old tissue. Occasionally such pieces were a mass of completely disintegrated cells with an intact tail

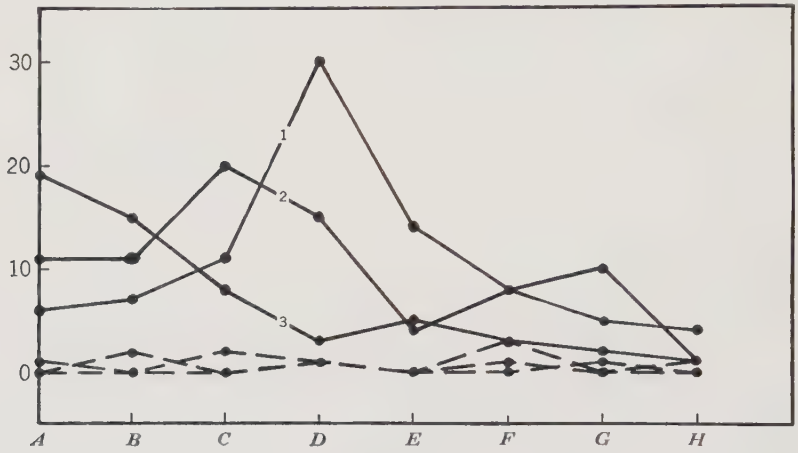


FIG. 21.—Death frequency in (1) M/100,000 strychnine sulphate, (2) M/300,000, (3) M/450,000, and controls (broken line). Ordinates are percentage dead and abscissae body-levels.

and a head in which the eyes were visible. Others were in a still further advanced stage of disintegration in which the type of head was not recognizable. The fact that pieces which had already developed normal heads were seen in various stages of disintegration shows that some, at least, of the pieces which die are not acephalic and suggests that in these experiments deaths ought not to be rated lower than acephalics. Consequently, head-frequency indices in these experiments were computed from living pieces only, and deaths were recorded and figured separately on a percentage basis.

In Figure 21, curves are given for percentages of deaths in the three concentrations used on 1/8 pieces. The deaths in M/300,000 were irregular. However, in continuous exposure to M/450,000 (3, Fig. 21), the largest number of deaths (19 per cent) occurred at the *A* level, and progressively fewer were recorded for more posterior levels. This shows the direct lethal action of this concentration and period of exposure in which the anterior levels are the most susceptible.

In short-time exposure to M/100,000 (1, Fig. 21) the opposite effect was seen. Here deaths were fewest in the *A* pieces (8 per cent) and most in the *D* pieces (27 per cent). This may be attributed to differential recovery in water from the effects of the treat-

ment. That is, the *A* pieces were able to tolerate the solution during the exposure and recover after return to water, but some of the *D* pieces were unable to do so, and consequently the death-rate was higher at the *D* level. Another important factor is the different degree of stimulation following section shown by pieces from various levels. Observations by Child (1914*a*) show that when pieces are placed in potassium cyanide immediately following section the *D* pieces are more susceptible than the *A* pieces. On the other hand, if exposure is made 48 hours after cutting, the reverse is true. Tests of carbon dioxide production (Robbins and Child, 1920) and oxygen consumption (Hyman, 1923) also show that *D* pieces are stimulated by section to a greater extent than *A* pieces. Since all exposures to strychnine were made immediately after cutting, the disintegrating spots in *D* pieces, with temporary exposure to high concentration, and their higher death-rate may have resulted from the greater stimulation of *D* pieces by cutting.

TABLE 4

MEAN TIMES IN HOURS TO APPEARANCE OF EYES IN 3/8 PIECES OF 16-MM. WORMS

Level of Piece	*ABC* (3/8)			*BCD* (3/8)			*CDE* (3/8)			*DEF* (3/8)		
Solutions	M/100,000	H_2O	M/450,000	M/100,000	H_2O	M/450,000	M/100,000	H_2O	M/450,000	M/109,000	H_2O	M/450,000
Mean of means of five series	80.6	77.7	79.8	79.7	78.2	82.2	84.4	81.7	81.6	88.7	83.9	86.1
P	0.0540		0.0330	0.3740		0.0126	0.0540		0.8512	0.0400		0.1036
Average standard deviations within lots	6.6	5.8	6.1	7.1	5.3	5.8	6.6	5.5	4.9	6.0	4.8	6.3
Rates	96.4	100.0	97.4	97.5	99.3	94.5	92.1	95.1	95.2	87.6	92.6	90.2

RATE OF EYE FORMATION

Observations of times of appearance of eyes were made on pieces long enough to produce normal heads as described earlier. In Table 4 are given the means of means and average standard deviations for the five series in each solution. The statistical significance (*P*, Table 4) shows that there is a real though slight delay in anterior pieces with continuous exposure and in posterior pieces with temporary exposure. This difference in reaction to the two solutions resembles the distribution of deaths just described. In both cases the continuous exposure to M/450,000 produced the greatest effect on the anterior pieces, and temporary exposure to M/100,000 showed the reverse situation.

Rates of development (Table 4) are calculated as described in the section on methods by expressing mean times as percentage values relative to the time of the *A* pieces in water. Such rates show a progressive decrease between adjacent levels with increasing distance from the head. This decrease is very regular in water and shows a general, though less regular, trend in the experimental solutions. Comparisons of the *ABC* with the *DEF* pieces in each solution show that the *DEF* pieces are significantly slower and

that the interval is greater in the strychnine solutions than in water. The significance (P) for this difference in M/100,000 is 0.0126; in M/450,000, 0.0148; and in water, 0.0300. The standard deviations for each lot are slightly smaller than those given by Watanabe (1935*a*) since observations were made at 8-hour intervals instead of at the 12-hour intervals which he used. The increase in variability from anterior to posterior levels which he reports was not found.

FISSION

Four experimental and four control lots of 50 12–14–mm. worms with heads removed were placed in 1 liter each of M/300,000 strychnine sulphate or well water. Two experimental and two control lots were kept in earthenware containers in order to determine whether slight irregularities of surface would tend to induce fission, and the other four were kept in glass crystallization dishes. All were covered and placed in darkness. Of the 200 worms in earthenware dishes 52 per cent in water and 2 per cent in strychnine underwent fission. In the glass dishes fissions occurred in 56 per cent in water and none in strychnine, making a total of 54 per cent fission in controls contrasted with only 1 per cent in the test solution. Instead of being increased, the number of fissions in controls in earthenware dishes is actually lower than in the glass dishes, but the difference is not significant. However, the very low number in experimental lots is striking evidence that strychnine inhibits fission even in headless animals.

DISCUSSION

Observations on the reactions of *E. dorotocephala* to strychnine treatment indicate that in this form the effects are intermediate between the depression exhibited by Protozoa and Coelenterata and the high degree of stimulation in the higher invertebrates and the vertebrates. Planarians in strychnine show the typical "reversal of inhibition" of antagonistic muscles, increased susceptibility to a rise in temperature, and greatly increased motor activity especially after touch, jarring, or exposure to light. However, they show no sudden contractions in response to such stimuli and no indication of the tetanus characteristic of strychnine action on higher forms. The increased activity is accompanied by loss of co-ordination and inability to adhere to the substratum similar to that observed by Hyman (1919*a*) on pieces in KCN and by Rulon (1936) in high concentrations of carbon dioxide. However, the incidence of fission differs greatly. In carbon dioxide (Rulon, 1936), in other anesthetics, and after removal of the head (Child, 1910, 1929) large numbers of fissions occur, but in strychnine only 1 per cent underwent fission even after decapitation as contrasted with 54 per cent in water. Obviously, the decreased motor co-ordination and inability to crawl normally are not sufficient to explain the lack of fission, for these occur also in carbon dioxide in which fissions are increased. It seems probable that the strychnine acts in some way to suppress the development of the fission plane.

In head-frequency experiments strychnine lots show a very striking increase over controls at all levels of the body. Evidence from this line of attack indicates that strychnine action on *Euplanaria* resembles that of depressants rather than that of stimulants. No evidence was found for preliminary stimulation followed by depression such as Hinrichs (1924) obtained with caffeine. The results with strychnine resemble most nearly those obtained with chloroform and ether, which are depressants particularly of the nervous system, rather than those obtained with chloretone and KCN, which are general proto-

plasmic depressants (Child, 1916; Buchanan, 1922). That is, agents which affect the protoplasm as a whole decrease head frequency in *A* pieces by direct inhibition of the cells at the anterior cut surface and cause only slight increase in *C* and *D* pieces, whereas agents acting principally on the nervous system cause little or no decrease in *A* pieces and greater increase in *C* and *D* by blocking the inhibiting effect of the posterior section. The unusual features of the strychnine series are the fact that no decreases but occasional increases were obtained in *A* pieces and the very high head-frequency indices at all levels of the body. It is possible that strychnine blocks the inhibiting factor more completely than do other agents and therefore causes greater increases in head frequency. Block produced by strychnine has been demonstrated with the unmedullated nerves of the walking legs of the crab, *Maia squinado* (Cowan and Ing, 1935). The similarity of the results with strychnine to those with depressants agrees with Knoefel's findings (1936) that the action of strychnine on peripheral nerve fibers is purely depression.

Experiments on delay of posterior or anterior section with short pieces cut immediately posterior to the mouth also give evidence concerning this posterior inhibiting factor. Control curves agree very closely with those of Child and Watanabe (1935) on *E. dorotocephala* and Watanabe (1935*b*) on *E. tigrina*. With delay of posterior section, head frequency increases very regularly from a low value with simultaneous anterior and posterior section to 100 per cent normal heads after 24 hours' delay. This short interval before maximal head frequency is obtained indicates that, once the cells at the anterior cut surface start forming a head, the posterior cut has little or no inhibiting effect. With delay of anterior section, however, a slightly different situation exists. Head-frequency values are low after simultaneous anterior and posterior cuts, but still lower after 24 hours' delay, and then gradually increase with increasing intervals to nearly all normal by 96 hours. This decrease at 24 hours occurs after the effect of cutting has disappeared as demonstrated by oxygen and carbon dioxide determinations (Hyman, 1919*a*, 1919*b*, 1923; Robbins and Child, 1920; Watanabe and Child, 1933). Consequently, it seems probable that the activity accompanying growth and differentiation at the posterior cut also plays a part in the inhibition. In strychnine, head-frequency values are high at all times in experiments with delay of anterior or posterior section. This indicates that strychnine acts on the piece in such a way as to block the inhibiting effect of the posterior cut and also of the growth and differentiation at the posterior surface.

Another very striking point is the unusually high percentage of biaxial forms appearing in the various experimental series. This formation of heads on both cut surfaces, instead of the normal head and tail development, is another evidence of the decrease or elimination by strychnine of correlative factors within the piece. In head-frequency experiments on 1/8 pieces of 16–mm. worms, the numbers of bipolar forms are low in pieces from anterior and posterior levels and reach a peak in *E* pieces. The slight decrease observed in *C* pieces (Fig. 7) was very regular, especially in temporary exposure to high concentration in which the numbers of bipolars were highest. This may be associated with the presence of the base of the pharynx in the *C* piece (Fig. 1). Since the pharynx contains a large amount of nervous tissue, the region at its anterior end may possibly be regarded as a secondary nervous center which may to some extent reinforce antero-posterior dominance.

The high frequency of bipolar heads at the *E* level is probably correlated with the fact that this is the region of the fission zone. This region appears to be a sort of neutral zone in which dominance in either direction is slight and probably variable, that is, the

cells of this region are to some degree physiologically isolated by their intermediate position from the dominance of both anterior and second zooids. In short pieces from this region there is little differential between anterior and posterior ends; consequently, anterior head frequency is high and bipolar head frequency is higher than at other body-levels. Even in controls short pieces from this level occasionally produce bipolar heads. Because of these characteristics the region of the fission zone appears to be a particularly favorable locus for production of bipolar heads, and in strychnine, which seems further to decrease correlative factors within the piece, the highest numbers occurred in this region. Since the fission zone varies somewhat in position in different individuals, and the longitudinal gradient is slight in the second zooid, both *D* and *F* levels show relatively high bipolar frequency but not as high as the *E* level.

The curve for counts of formative cells by Curtis and Schulze (1934) on *E. tigrina* shows an interesting parallelism to that found here for percentages of bipolar forms. Although a different species, *E. tigrina* is closely related to *E. dorotocephala* and is a fissioning form. The similarity in the two curves consists merely in that peaks occur in *B* and *E* levels. However, the peak for bipolar frequency is considerably higher at *E* than at *B*, whereas those for formative cells are nearly equivalent. Work in many lines has shown that the physiological factors of distance from the dominant region, presence or absence of a fission plane, inhibiting effect of a posterior cut surface, etc., are more fundamental in regeneration than are the morphological factors which have themselves been determined in their development by previously acting correlative factors. The large amount of parenchyma tissue may be favorable for bipolar production, but physiological conditions are believed to be fundamental.

Bipolar forms have been observed by other authors in various species of *Euplanaria*, but they were commonly produced only in short pieces. Morgan (1903) found "heteromorphic heads" in short pieces from the pharyngeal region of *E. tigrina*. Child (1911*b*) described various types of biaxial forms with one or two pharynges and with or without common lateral tails. Rustia (1925) studied the effects on bipolar production of a number of agents on *E. dorotocephala* and *E. tigrina*. He also found the highest numbers at the *B* level and at the fission zone, but he used very short (1-mm.) pieces which usually give higher numbers of bipolars than do longer pieces. J. A. Miller (in press, this *Journal*) has produced bipolar forms in *E. dorotocephala* as a result of induction by transplantation. No agents other than strychnine have been reported as producing large numbers of bipolar forms in pieces as long as 1/8 of 16-mm. worms.

In experiments on delay of posterior section the number of bipolar forms in both controls and strychnine series is very high. Morgan (1903) used a delayed section experiment to test his theory that a developing head on one end of the piece influenced the other end to form a head. He found fewer bipolars, if anything, with delayed than with simultaneous cuts, but he did not have enough cases to be significant. Abeloos (1927, 1930) found that 3 days' delay of posterior section in short pieces of *Planaria gonocephala* gave approximately 1/4 bipolar forms, whereas a similar delay of anterior section produced no bipolars. In Watanabe's experiments on *E. tigrina* (1935*b*) a few bipolars appeared in 1/8 pieces from the fission zone and in delayed section experiments, but their distribution in relation to the interval of delay was irregular. In all, 52 bipolars appeared in experiments with delay of posterior section and only 17 with anterior delayed. There were also a few in the partial section experiments, especially with simultaneous anterior section and section of posterior lateral regions with delay of section of nerve cords, and

also in experiments in which one section was made under anesthesia. Since the frequencies in all cases were low, he did not attempt any conclusions with regard to their distribution. However, they seem to agree well with the experiments here reported in which larger numbers occurred.

In the controls for the strychnine series, bipolar frequency increased regularly with delay of posterior section but decreased with delay of anterior section. This increase is probably related to the fact that, when the anterior cut is made, the piece is isolated from the old head and the fission plane begins to develop. When the posterior cut is made later, this region is more active and ready to form a head. The longer the delay (up to 18 hours in these experiments) the more pieces produce heads at the posterior surface and result in bipolar forms. That the bipolar production is related to the development of the fission plane is indicated by the fact that many of the controls fissioned after the first cut was made and could not be used. Also, unpublished experiments with recently collected worms which had undergone fission and had short tails gave very few bipolar forms after delayed posterior section, suggesting that the lack of development of the fission zone was responsible. The decrease in bipolarity with delayed anterior section probably means that, during the period of development of the posterior surface under the influence of the old head, the regenerating tissue is determined as a tail and the resulting piece when later isolated is unipolar. The longer the interval of this influence, the more definite is the determination.

The occurrence of tailless forms has also been observed by Child (1912) in alcohol, KCN, low temperature, and ether. The experiments suggest that in strychnine the failure to develop tails is caused not by direct inhibition or increased scale of organization but rather by incomplete reversal of polarity. Observations of posterior regeneration in 1/8 pieces in M/100,000 strychnine sulphate (Table 2) and in M/500,000 with delay of posterior section (Table 3) reveal a continuous series from normal tails (representing no reversal) through short tails, no outgrowth, and inhibited head-forms (partial reversal) to normal heads (complete reversal). The cases of no posterior outgrowth are intermediate and represent either inhibited heads or inhibited tails, as demonstrated by the fact that some show reversal of ciliary beat at the posterior healed surface and some do not.

The experiments on rate of eye development were carried out as a means of testing whether the marked increase in head frequency in strychnine series was caused by an acceleration of development of the cells at the anterior cut surface or on the piece as a whole. Since the only difference in time was a very slight delay, it is concluded that strychnine does not accelerate head development, but decreases or eliminates the correlative factor resulting from posterior section which tends to prevent head development. The gradient in rate at different levels of the body, was in general, similar to that reported by Watanabe (1935*a*), though the differences between levels were not as great. It seems probable that this difference is related to the fact that the experiments were carried on at different temperatures. At the higher temperatures used by Watanabe (24°–28° C. as contrasted with 18°–22° C.) the rate was increased more in anterior than in posterior pieces. This was also seen in Rulon's data (1936) in which the controls of one series at 32° showed a great difference in rate between anterior and posterior pieces, whereas the other experiment at 20° showed a relatively small difference. In series treated with acids Rulon reports a considerably greater retardation, especially in anterior pieces, than that seen in strychnine. This suggests that the H-ion causes a more general protoplasmic inhibition than the strychnine, in which the effect is practically entirely on the factor inhibiting or

preventing head development. The fact that there is a small but consistent delay in strychnine indicates a certain amount of inhibitory action on the head-forming cells, but this appears to be very slight. Certainly, no stimulation was observed that could account for the great increases in head frequency and bipolarity.

SUMMARY

1. Individuals of the species *E. dorotocephala* in solutions of strychnine sulphate show greatly increased activity, spasmodic contractions, and poorly co-ordinated locomotion. They do not exhibit feeding, rheotactic, or righting reactions, and they are easily dislodged from the substratum. They show the reversal of inhibition of antagonistic muscles which is characteristic of strychnine action on annelids and arthropods but not the tetanus exhibited by vertebrates.

2. With 1/8 pieces of 16-mm. *E. dorotocephala* the head frequencies obtained by treatment with various concentrations of strychnine sulphate are consistently higher than those obtained with well-water controls.

3. In short pieces (1.5 mm.) taken from immediately posterior to the mouth, controls give very low head frequencies after simultaneous anterior and posterior section and increasing values with increasing intervals of delay of either section. Maximal indices were obtained after 24 hours' delay of posterior section and after 96 hours' delay of anterior section. In strychnine all lots gave very high values with maximum after 8 hours' delay of posterior section and 48 hours' delay of anterior section.

4. Large numbers of bipolar forms appear in 1/8 and shorter pieces in strychnine-treated lots. The largest numbers occur in the *E* pieces which are cut from behind the mouth. After 16-hour exposure to M/100,000 strychnine sulphate, 31.4 per cent of living 1/8 pieces from this level developed bipolar forms. Fewer appeared in the lower concentrations and none in water.

5. With increasing intervals of delay of posterior section numbers of bipolars formed in control groups increased from 1.1 per cent with no delay to a maximum of 26.1 per cent after 18 hours' delay. In strychnine large numbers occurred in all groups with a maximum of 73.8 per cent after 12 hours' delay of the posterior section.

6. Large numbers of tailless pieces also occurred in experimental lots. In 1/8 pieces these were most numerous in pieces cut from the anterior end of the worm. Evidence is presented that some tailless pieces, particularly from behind the mouth, were probably bipolar forms with one completely inhibited head.

7. In 1/8 pieces with continuous exposure to M/450,000, deaths were most frequent in the *A* pieces and decreased posteriorly. After short-time exposures to M/100,000, however, the deaths increased from *A* to *D* levels and then decreased.

8. In 3/8 pieces at 18°–22° C. rates of eye-pigment spot formation decreased with increasing distance of level of head development from the head of the original animal. Experimental lots exposed to M/100,000 or M/450,000 strychnine sulphate showed slightly slower eyespot formation than did well-water controls. Calculations of statistical probabilities show that the delay was more significant in anterior pieces with continuous exposure and in pharyngeal pieces with temporary exposure. No acceleration was observed.

9. In decapitated 12–14-mm. worms in M/300,000 strychnine only 1 per cent underwent fission, whereas in controls fissions were recorded in 54 per cent.

10. The data presented show that the effect of strychnine on head frequency resembles that of certain inhibiting agents such as chloroform and ether with the exception

that no decrease in head frequency occurred in *A* pieces. The observations of rates of eye development, head frequency, bipolar frequency, and effects of delayed section lead to the conclusion that the results are due to the decrease or obliteration of the effect of the posterior section rather than to the stimulation of the head-forming cells.

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